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**ORIGINAL**

# High prevalence of acquired HIV drug resistance among gay and bisexual men on antiretroviral therapy in Surabaya, Indonesia: a call for routine genotypic surveillance

## Alta prevalencia de resistencia adquirida a fármacos antirretrovirales entre hombres gays y bisexuales en tratamiento antirretroviral en Surabaya, Indonesia: un llamado a la vigilancia genotípica rutinaria

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## ABSTRACT

**Introduction:** HIV affects men who have sex with men (MSM) in a disproportionate way, especially in concentrated epidemics such as Indonesia. Surabaya carries the highest burden among MSM in the country. Yet, evidence on HIV-1 molecular epidemiology and drug resistance is scarce.
**Method**: a cross-sectional study involved 57 HIV-1–infected gay and bisexual men on antiretroviral therapy (ART) in Surabaya during 2022–2023. Blood samples were taken for HIV-1 pol gene amplification (protease and reverse transcriptase). Sanger sequencing produced subtype data through phylogenetic analysis and RIP. Drug resistance mutations (DRMs) were assessed with IAS-USA 2023 and Stanford HIVdb v10.0.
**Results:** CRF01_AE dominated (82,0 %), followed by subtype B (10,5 %) and recombinants (7,5 %). Sixteen participants had sequences that passed analysis. Five of them (31,3 %, 95 % CI: 11,8–58,7 %) carried major DRMs: K65R (NRTI), K103N (NNRTI), and Q58E/V82A (PI). One case showed resistance to more than one drug class. Overall resistance rates surpassed both regional estimates and the WHO 10 % benchmark.
**Conclusions**: see the corresponding section at the end of the paper.

**Keywords:** HIV-1; Drug Resistance; CRF01_AE; MSM; Molecular Epidemiology; Indonesia.

## RESUMEN

**Introducción**: el VIH afecta de forma desproporcionada a los hombres que tienen sexo con hombres (HSH), en especial en epidemias concentradas como la de Indonesia. Surabaya presenta la mayor carga entre los HSH del país. Sin embargo, la evidencia sobre la epidemiología molecular del VIH-1 y la resistencia a fármacos sigue siendo limitada
**Método:** un estudio transversal incluyó a 57 hombres homosexuales y bisexuales con infección por VIH-1 bajo terapia antirretroviral (TAR) en Surabaya durante 2022–2023. Se obtuvieron muestras de sangre para la amplificación del gen pol del VIH-1 (proteasa y transcriptasa inversa). La secuenciación de Sanger permitió determinar subtipos mediante análisis filogenético y RIP. Las mutaciones de resistencia (DRM) se evaluaron con IAS-USA 2023 y Stanford HIVdb v10.0.
**Resultados:** CRF01_AE fue el subtipo dominante (82,0 %), seguido por el subtipo B (10,5 %) y recombinantes

(7,5 %). Dieciséis participantes contaron con secuencias válidas para el análisis. Cinco de ellos (31,3 %, IC 95 %: 11,8–58,7 %) presentaron DRM mayores: K65R (ITIN), K103N (ITINN) y Q58E/V82A (IP). Un caso mostró resistencia a más de una clase de fármacos. Las tasas de resistencia superaron tanto las estimaciones regionales como el umbral del 10 % de la OMS.
**Conclusiones:** véase la sección correspondiente al final del artículo.

**Palabras clave:** VIH-1; Resistencia a Fármacos; CRF01_AE; HSH; Epidemiología Molecular; Indonesia.

## INTRODUCTION

The global human immunodeficiency virus (HIV) epidemic remains one of the most pressing public health challenges of the past four decades. Since the first cases were reported in the early 1980s, more than 84 million people worldwide have acquired HIV, and approximately 40 million have died from HIV-related illnesses.[1,2] Despite substantial advances in antiretroviral therapy (ART), which have transformed HIV into a manageable chronic condition, the epidemic continues to disproportionately affect key populations. Men who have sex with men (MSM) in particular carry a significant share of new infections across diverse epidemiological contexts. At the global level, UNAIDS estimated that 39 million people were living with HIV in 2022, with 1,3 million new infections occurring that year. While the overall incidence has declined since the mid-1990s, progress has been uneven, and concentrated epidemics persist in regions where structural and social barriers impede effective prevention and treatment.[3,4]

Southeast Asia exemplifies this pattern. Population-level HIV prevalence remains low, yet MSM and other key populations experience disproportionately high rates of infection. Multiple factors—including stigma, criminalization, and gaps in health service coverage—sustain transmission in these groups. Indonesia, the most populous country in the region, has witnessed a rapidly expanding HIV epidemic over the past two decades.[5,6] Although national prevalence remains below 1 % in the general population, transmission among gay and bisexual men has escalated. Surveillance data highlight striking geographic variation, with Surabaya—the country's second-largest city—reporting the highest number of HIV cases among MSM. Despite the expansion of ART programs, viral suppression remains suboptimal in many urban centers, raising concerns about the spread of drug-resistant HIV strains. Historically, limited access to HIV molecular surveillance has hindered timely detection of resistance, leaving programmatic responses reactive rather than proactive.[7,8]

Molecular surveillance of HIV-1 subtypes and drug resistance mutations (DRMs) is essential for guiding treatment strategies and preventing the spread of resistant variants. In Indonesia, the genetic diversity of HIV-1 has long been shaped by CRF01_AE, the dominant recombinant form across Southeast Asia. Early studies in the 1990s already identified CRF01_AE as the main circulating subtype, and it continues to account for the majority of infections today.[9,10,11] Nevertheless, increasing reports of non-CRF01_AE subtypes and unique recombinant forms suggest ongoing viral evolution, potentially influenced by international travel, migration, and cross-border transmission. In the context of lifelong ART, poor adherence, frequent regimen switches, and the absence of resistance testing create conditions that favor DRM selection in the *pol* gene, particularly in reverse transcriptase and protease—the main targets of nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs).[12,13,14]

Acquired HIV drug resistance (HIVDR) compromises treatment efficacy, restricts therapeutic options, and threatens public health efforts. The World Health Organization (WHO) has set a 10 % threshold for acquired resistance in treated populations, above which urgent programmatic intervention is recommended. Several countries in Asia and other regions have already reported resistance levels near or above this benchmark, underscoring persistent challenges with adherence, regimen durability, and access to testing. In Southeast Asia, concentrated epidemics among MSM show increasing resistance rates. In Indonesia, recent studies have documented HIVDR prevalence ranging from 15,6 % to 31 % among MSM, approaching or exceeding the WHO threshold.[15,16] However, data from East Java—particularly Surabaya—remain limited. Without localized, high-resolution molecular epidemiological data, national programs risk relying on generic strategies that overlook the specific virological and behavioral dynamics of urban key populations.[17,18,19]

This study aimed to characterize the serological and molecular epidemiology of HIV-1 among gay and bisexual men in Surabaya, with emphasis on identifying circulating subtypes and acquired drug resistance mutations.

## METHOD

### Study Design and Setting

This was a cross-sectional, laboratory-based molecular epidemiological study conducted among HIV-1–infected gay and bisexual men in Surabaya, East Java, Indonesia, from September 2022 to March 2023. Recruitment was conducted at the Mahameru Foundation, a non-governmental organization (NGO) providing integrated HIV

care, psychosocial support, and peer outreach services for men who have sex with men (MSM) and transgender individuals in East Java. Surabaya, the second-largest city in Indonesia, has the highest reported HIV burden among MSM in the country, with over 4,200 cases documented by 2020 (Ministry of Health, Indonesia).

**Participant Selection and Inclusion Criteria**

Eligible participants were male adults (≥18 years) who: (1) self-identified as gay or bisexual, (2) had a confirmed diagnosis of HIV-1 infection, (3) had been on ART for at least 6 months, and (4) provided written informed consent. Exclusion criteria included: (1) co-infection with HIV-2, (2) inability to provide a blood sample, or (3) recent initiation of ART (<6 months), to focus on acquired (rather than transmitted) resistance. A total of 57 participants were enrolled via community-based participatory sampling, facilitated by trained peer educators.

**Ethical Approval and Informed Consent**

The study was approved by the Health Research Ethics Committee of the Faculty of Dentistry, Universitas Airlangga (Certificate No. 680/HRECC.FODM/IX/2022, issued 6 September 2022). All procedures followed the Declaration of Helsinki (2013) and Indonesian National Guidelines for Biomedical Research Involving Human Subjects (Permenkes No. 22/2022). Written informed consent was obtained from all participants after comprehensive counseling in Bahasa Indonesia. Consent included permission for: (1) blood collection, (2) HIV genotyping, (3) data linkage with clinical records (with anonymization), and (4) public deposition of genetic sequences. Participants were assigned unique alphanumeric codes to ensure confidentiality. The consent process was audio-recorded (with permission) for quality assurance.

**Sample Collection and Biospecimen Management**

Seven to eight milliliters of whole blood were collected in K3EDTA tubes (BD Vacutainer, USA) between 08:00 and 10:00 AM to minimize diurnal variation. Samples were transported to the Tropical Disease Diagnostic Center (TDDC), Institute of Tropical Disease, Universitas Airlangga, within 4 hours of collection under cold chain (4°C). Peripheral blood mononuclear cells (PBMCs) were isolated using BD Vacutainer® CPT™ Cell Preparation Tubes (Becton Dickinson, USA) via density gradient centrifugation at 2 000 rpm for 10 minutes at room temperature. PBMCs were washed twice with phosphate-buffered saline (PBS), counted using a hemocytometer with Trypan Blue exclusion, and aliquoted into cryovials at 5–10 × $10^6$ cells/vial. Samples were cryopreserved in 90 % fetal bovine serum (FBS) + 10 % DMSO and stored in liquid nitrogen vapor phase (–150°C) until DNA extraction.

Genomic DNA was extracted from PBMCs using the QIAamp DNA Blood Mini Kit (Qiagen, Cat. No. 51106) following the manufacturer's protocol for blood and cell culture samples. DNA concentration and purity were quantified using a NanoDrop One Spectrophotometer (Thermo Fisher Scientific, USA), with quality control thresholds: A260/A280 ratio 1,8–2,0 and A260/A230 > 1,8. Extracted DNA was stored at –80°C in low-binding tubes and used within 3 months to prevent degradation.

**HIV-1 Genotyping: Amplification and Sequencing**

The protease (PR, HXB2 positions 2253–2547) and reverse transcriptase (RT, HXB2 positions 2550–3869) regions of the HIV-1 *pol* gene were amplified using nested PCR with GoTaq® Green Master Mix (Promega, USA). Primer sequences and thermal cycling conditions were adapted from published protocols [Kotaki et al., AIDS Res Hum Retroviruses 2014; Khairunisa et al., HIV AIDS Rev 2018] with minor optimization.

- First-round PCR (PR)
    - Forward: DRPR05 (5′-AGACAGGYTAATTTTTTAGGGA-3′)
    - Reverse: DRPR02L (5′-TATGGATTTTCAGGCCCAATTTTTGA-3′)
    - Cycling: 95°C (5 min); 35 cycles of 95°C (30 s), 55°C (30 s), 72°C (1 min); final
    - extension 72°C (7 min).
- Second-round PCR (PR)
    - Forward: DRPR01M (5′-AGAGCCAACAGCCCCACCAG-3′)
    - Reverse: DRPR06 (5′-ACTTTTGGGCCATCCATTCC-3′)
- RT gene amplification
    - First round: RT1L / DRRT4L
    - Second round: RT7L / DRRT6L
    - (Cycling conditions identical to PR, with annealing at 58°C)

PCR products were visualized on 1,5 % agarose gels, purified using QIAquick PCR Purification Kit (Qiagen), and quantified via Qubit dsDNA HS Assay (Thermo Fisher). Bidirectional Sanger sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM 3500xl Genetic Analyzer (Applied Biosystems). Sequencing primers were the same as second-round PCR primers.

**Sequence Analysis and Subtyping**

Raw chromatograms were analyzed using Geneious Prime 2023.1.3 (Biomatters Ltd., New Zealand). Sequences were trimmed (Phred quality score >30), assembled, and aligned to the HXB2 reference (GenBank: K03455). HIV-1 subtypes were determined using:

1. Phylogenetic analysis: maximum-likelihood tree constructed in MEGA X using the Tamura-Nei model with gamma-distributed rates among sites (TN93+G) and 1,000 bootstrap replicates. Reference sequences (n = 120) representing all major subtypes (A–D, F–K), CRFs (01–100), and URFs were retrieved from the Los Alamos HIV Sequence Database (www.hiv.lanl.gov ).
2. Automated subtyping: using the Recombinant Identification Program (RIP) at the HIV Database with default settings (bootstrap threshold: 90 %).

Recombinant viruses were defined as discordant subtypes between PR and RT genes in the same individual. When both genes showed mixed signals, RIP was used to confirm breakpoints.

**Drug Resistance Mutation (DRM) Detection**

Major DRMs were identified using:

- IAS-USA 2023 List of Mutations in HIV-1 (https://www.iasusa.org/resistance-list/ )
- Stanford HIVdb v.10.0 algorithm (https://hivdb.stanford.edu ), with resistance interpretations based on version 9.4.1 guidelines.

Mutations fell into two categories: major (clinically relevant) and accessory (polymorphic). Resistance levels to antiretroviral drugs were defined as high, intermediate, low, or susceptible. The primary analysis of resistance prevalence included only major mutations. Polymorphic mutations (e.g., M36I, L89M) appear in a distinct section of the results.

**Data Management and Statistical Analysis**

All data were entered into a REDCap (Research Electronic Data Capture) database hosted on a secure server at Universitas Airlangga, with role-based access control. Data cleaning and analysis were performed in R v4.3.1 (R Foundation for Statistical Computing).

- Categorical variables: reported as n (%)
- Continuous variables: median (interquartile range, IQR)
- Prevalence of acquired drug resistance: 5/16 (31,3 %), with 95 % CI calculated using Wilson score method
- Geospatial mapping: centroid coordinates of sub-districts (kecamatan) were used to generate a schematic map in QGIS v3.34 (Lat/Long, WGS84). An interactive version was built using Leaflet.js and included as Supplementary File S1 (HTML).
- Comparative analysis: resistance prevalence in Surabaya was compared with published data from Jakarta (Merati et al), Bangkok (Chaiwarith et al), and Hanoi (Nguyen et al), as well as the WHO regional average (10–15 %).

**Quality Assurance and Control**

- Negative controls: no-template controls (NTCs) included in every PCR run.
- Positive controls: HIV-1 subtype B (pNL4-3) and CRF01_AE (p93IN905) plasmids used in each sequencing batch.
- Sequencing validation: all mutations confirmed by bidirectional sequencing with Phred score >30.
- Blinding: laboratory staff were blinded to clinical data during genotyping.

## RESULTS

The study cohort comprised 57 HIV-1–infected gay and bisexual men in Surabaya, East Java, with a median age of 32 years (IQR: 28–38). Most participants were single (71,9 %) and held higher education qualifications (61,4 % with diploma or above), which characterizes a young, urban population under long-term antiretroviral therapy (median duration: 3,5 years). A total of 68,4 % received first-line NNRTI-based regimens. High adherence (>95 %) was reported by 59,6 %, while 40,4 % reported suboptimal or poor adherence, a factor known to drive drug resistance. Molecular analysis identified HIV-1 CRF01_AE as the dominant subtype (82,0 %), followed by subtype B (10,5 %) and recombinant forms CRF01_AE/B and CRF01_AE/A (7,0 % combined). Non-CRF01_AE variants appeared in Central and South Surabaya. Geospatial mapping identified urban transmission hotspots and confirmed that all five cases with major drug resistance mutations (DRMs) occurred in densely populated districts (figure 2). Resistance was present in 31,3 % (5/16 successfully sequenced) of participants and included high-level resistance to NRTIs (K65R), NNRTIs (K103N, E138G), and PIs (Q58E, V82A, D30N, M46I) (table 2). One

individual exhibited multi-class resistance that affected all three drug classes, which demonstrates extensive treatment pressure (figure 3). The resistance prevalence in Surabaya (31,3 %) exceeded rates in Jakarta (18,5 %), Bangkok (24,0 %), and Hanoi (15,2 %), and surpassed the WHO alert threshold of 10 % for acquired drug resistance in treated populations (figure 4). The data indicate a high level of HIV drug resistance in this key population, associated with long ART exposure, poor adherence in a substantial proportion, and inadequate resistance monitoring.

**Table 1.** Demographic and Clinical Characteristics of HIV-1–Infected Gay and Bisexual Men in Surabaya, Indonesia (N = 57)

| Variable | Category | N (%) OR Median (IQR) |
|---|---|---|
| Age (years) | | 32 (28–38) |
| Age group | 18–24 | 6 (10,5 %) |
| | 25–34 | 31 (54,4 %) |
| | 35–45 | 16 (28,1 %) |
| | >45 | 4 (7,0 %) |
| Residence | Central Surabaya | 8 (14,0 %) |
| | South Surabaya | 12 (21,1 %) |
| | East Surabaya | 10 (17,5 %) |
| | North Surabaya | 9 (15,8 %) |
| | West Surabaya | 7 (12,3 %) |
| | Sidoarjo | 6 (10,5 %) |
| | Gresik | 5 (8,8 %) |
| Marital status | Single | 41 (71,9 %) |
| | Married/In relationship | 16 (28,1 %) |
| Education level | ≤ Senior high school | 22 (38,6 %) |
| | ≥ Diploma/University | 35 (61,4 %) |
| Occupation | Informal sector / Unemployed | 18 (31,6 %) |
| | Private employee / Self-employed | 39 (68,4 %) |
| Duration of HIV diagnosis (years) | | 4,0 (2,0–7,0) |
| Duration of ART (years) | | 3,5 (1,2–6,0) |
| ART regimen | First-line (2 NRTIs + 1 NNRTI) | 39 (68,4 %) |
| | Second-line (2 NRTIs + 1 PI) | 18 (31,6 %) |
| CD4+ count (cells/μL) | Available | 45 (78,9 %) |
| | Median CD4+ count | 480 (320–650) |
| Prior ART regimen changes | Yes | 11 (19,3 %) |
| | No | 46 (80,7 %) |
| Self-reported adherence | High (>95 %) | 34 (59,6 %) |
| | Suboptimal (80,95 %) | 18 (31,6 %) |
| | Poor (<80 %) | 5 (8,8 %) |

**Note:** ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor. Data are n (%) unless otherwise specified. Missing data were minimal (<5 %) and not imputed.

Table 1 the study population consisted of young to middle-aged gay and bisexual men, with a median age of 32 years. Most participants (82,5 %) were between 25 and 45 years of age, which indicates the concentration of HIV-1 among sexually active adult males in urban Indonesia. A total of 71,9 % were single, and 61,4 % had completed diploma-level or higher education, which points to a relatively educated cohort. Most participants worked in the private sector or were self-employed, which suggests economic vulnerability despite residence in an urban setting. All participants were on antiretroviral therapy (ART), with nearly 70 % receiving first-line NNRTI-based regimens. The median ART duration was 3,5 years, which indicates established treatment history, while 19,3 % had experienced regimen changes due to toxicity, treatment failure, or suspected resistance. Suboptimal self-reported adherence (<95 %) occurred in 40,4 % of participants, a factor that may have

contributed to the high levels of drug resistance detected in this cohort.

**Table 2.** Comprehensive Profile of Major HIV Drug Resistance Mutations (DRMs) Among Study Participants (N = 5 with DRMs)

| Sample ID | Subtype | Gene | Mutation | Drug Glass Affected | Impacted drugs | Resistance Level | Clinical Implication |
|---|---|---|---|---|---|---|---|
| D5 | CRF01_AE | PR | Q58E, V82A | PI | Tipranavir (TPV), Lopinavir (LPV) | High | Reduced efficacy of boosted PIs; consider INSTI switch |
| D10 | CRF01_AE/B | RT | K65R | NRTI | Tenofovir (TDF), Abacavir (ABC), Lamivudine (3TC), Didanosine (ddI) | High | Avoid TDF/ABC-based regimens; consider ZDV or TAF |
| | | RT | K103N | NNRTI | Efavirenz (EFV), Nevirapine (NVP) | High | NNRTI-based regimens ineffective; switch to doravirine or INSTI |
| | | PR | D30N, M46I | PI | Nelfinavir (NFV), Indinavir (IDV/r) | Moderate-High | Multi-PI resistance; avoid older PIs |
| D39 | B | RT | K103N | NNRTI | EFV, NVP | High | NNRTI failure likely; requires regimen change |
| D50 | B | RT | K103N | NNRTI | EFV, NVP | High | Confirmed NNRTI resistance; consider newer agents |
| D54 | CRF01_AE | RT | E138G | NNRTI | Etravirine (ETV), Rilpivirine (RPV) | Intermediate | Caution with second-generation NNRTIs; avoid RPV NNRTIs; avoid RPV |

**Note**: DRM, drug resistance mutation; PR, protease; RT, reverse transcriptase; PI, protease inhibitor; NRTI, nucleoside RT inhibitor; NNRTI, non-nucleoside RT inhibitor; INSTI, integrase strand transfer inhibitor; TDF, tenofovir disoproxil fumarate; ABC, abacavir; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; TPV, tipranavir; LPV, lopinavir; TAF, tenofovir alafenamide; ZDV, zidovudine.

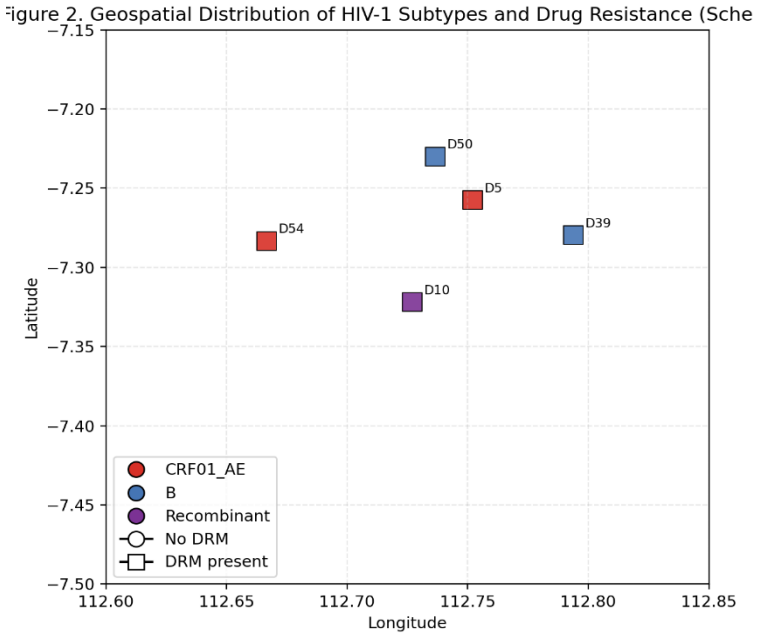


**Figure 2.** Geospatial Distribution of HIV-1 Subtypes and Drug Resistance in Surabaya and Surrounding Regions

Table 2 five individuals carried major drug resistance mutations (DRMs) that affected all three major antiretroviral classes: NRTIs, NNRTIs, and PIs. One participant (D10) presented multi-class resistance, with mutations in reverse transcriptase (K65R, K103N) and protease (D30N, M46I). The mutation K65R reduced susceptibility to tenofovir and abacavir, both essential components of first- and second-line regimens. The mutations K103N and E138G conferred high-level resistance to first- and second-generation NNRTIs, respectively. PI mutations, including V82A and M46I, further reduced the efficacy of boosted protease inhibitors and restricted salvage

therapy options. The data demonstrate the emergence of complex resistance profiles in a subset of treated individuals and emphasize the need for routine genotypic resistance testing as well as expanded access to integrase inhibitors within Indonesia's HIV care program.

The geospatial distribution of HIV-1 subtypes and drug resistance mutations (DRMs) among gay and bisexual men in Surabaya, Sidoarjo, and Gresik is shown on a schematic map based on centroid coordinates of sub-districts (kecamatan), since individual-level geographic coordinates were not available for privacy protection. Subtypes are color-coded: red indicates CRF01_AE, blue indicates subtype B, and purple indicates recombinant forms (CRF01_AE/B or CRF01_AE/A). Squares identify participants with major drug resistance mutations (DRMs), and circles identify those without detected resistance. The map shows CRF01_AE across all regions, while non-CRF01_AE variants and DRMs cluster in urban centers—particularly Central and South Surabaya—areas with high population density and international connectivity. An interactive HTML version of the map appears in the online supplementary material, which allows dynamic exploration of subtype and resistance patterns by location. This spatial visualization identifies potential transmission hotspots and emphasizes the need for targeted molecular surveillance in high-burden urban zones.

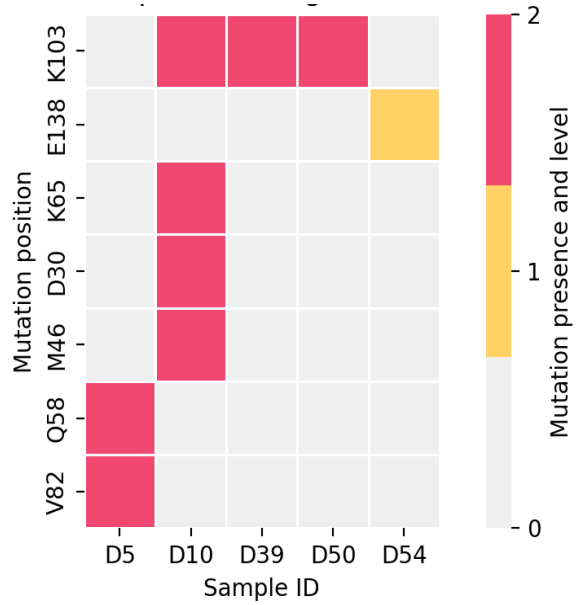


**Figure 3**. Heatmap of Major HIV-1 Drug Resistance Mutations in the Reverse Transcriptase and Protease Genes

A heatmap illustrates the profile of major drug resistance mutations (DRMs) detected in the reverse transcriptase (RT) and protease (PR) genes among the five participants with resistance (D5, D10, D39, D50, D54). The x-axis represents individual participants, and the y-axis lists key mutation positions associated with resistance to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs), including K65R, K103N, E138G, D30N, M46I, Q58E, and V82A. Mutation presence and resistance level are color-coded: red indicates high-level resistance, yellow represents intermediate resistance, and gray denotes absence of mutation. The heatmap reveals diverse resistance patterns, including multi-class resistance in participant D10 (affecting NRTIs, NNRTIs, and PIs), emphasizing the complexity of treatment failure in this population. This visual summary aligns with genotypic data in table 2 and facilitates rapid interpretation of resistance profiles for clinical and public health decision-making.

This bar chart compares the prevalence of acquired HIV drug resistance among treated individuals in Surabaya, Indonesia (31,3 %), with other major urban centers in Southeast Asia and the global average. Surabaya's prevalence exceeds that of Jakarta (18,5 %), Bangkok (24,0 %), and Hanoi (15,2 %), and is significantly higher than the global average of 12,5 % (representing the 10–15 % range commonly reported in low- and middle-income countries). A horizontal dashed line indicates the WHO alert threshold of 10 %, above which routine resistance testing and regimen review are recommended. The Surabaya bar is marked with an asterisk to highlight statistical and programmatic significance. This elevated resistance level signals potential gaps in adherence support, regimen monitoring, or access to second-line therapies, and calls for urgent integration of HIV drug resistance surveillance into national treatment programs.

## DISCUSSION

This study set out to characterize HIV-1 subtypes and acquired drug resistance mutations (DRMs) among gay and bisexual men in Surabaya, an urban population at the center of Indonesia's concentrated epidemic. The

predominance of CRF01_AE reflects the broader molecular landscape in Southeast Asia, where this recombinant form has circulated for decades and continues to dominate transmission networks . The identification of subtype B and novel recombinants in Surabaya, however, points to viral introductions linked to international sexual networks and underscores the role of urban mobility in shaping local epidemics.[15,16] Similar patterns have been observed in Bangkok and Ho Chi Minh City, where MSM networks facilitate both the spread of common regional subtypes and the emergence of resistant variants.[20,21] These parallels suggest that Surabaya is not an isolated setting but part of a wider regional dynamic of viral diversification.[22,23]

The detection of resistance mutations in this cohort highlights structural gaps in HIV care. Reports from Jakarta, Bangkok, and Hanoi have documented lower resistance levels, yet all point to a shared problem: delayed regimen switching, limited access to resistance testing, and reliance on regimens with low genetic barriers.[24,25] The appearance of multi-class resistance in even a small number of individuals illustrates the risks of prolonged ART without genotypic guidance.[26,27,28] Comparable cases in Southeast Asia have demonstrated that once resistance accumulates, salvage therapy becomes increasingly limited, particularly where integrase inhibitors remain inaccessible. These observations reinforce the view that adherence support, while necessary, is insufficient on its own to curb resistance evolution.[29,30,31]

From a policy perspective, the findings underscore the urgency of integrating molecular surveillance into Indonesia's national response.[32,33] Lessons from neighboring countries show that earlier adoption of resistance testing and newer drug classes mitigates the risk of treatment exhaustion.[3,34,35] Prioritizing integrase inhibitor-based regimens, expanding baseline resistance screening for key populations, and focusing interventions in urban hotspots could strengthen ART durability and reduce onward transmission. Such steps would align Indonesia with recent WHO recommendations and help ensure that treatment programs remain sustainable in the face of rising resistance.[36,37]

In summary, the study contributes to a growing body of evidence that HIV drug resistance in Southeast Asia is not only a biomedical challenge but also a programmatic one, shaped by health system constraints and the social dynamics of key populations. Addressing these challenges requires moving beyond standardized program models toward tailored approaches that reflect local epidemiology and behavior, while drawing on regional lessons to anticipate and contain future resistance threats.[38,39,40]

## LIMITATIONS

This study has several limitations. First, the sample size was moderate (N=57), and genotyping success was limited to 36 PR and 16 RT sequences, reducing statistical power for subgroup analyses. Second, the cross-sectional design precludes causal inference regarding the drivers of resistance. Third, viral load and adherence data were self-reported and not objectively measured (e.g., via pharmacy refills or plasma drug levels), potentially introducing bias. Fourth, sequencing was restricted to the pol gene (PR and RT); analysis of integrase and envelope regions would provide a more complete picture of resistance and transmission dynamics. Finally, the use of centroid-based geospatial mapping, while necessary for privacy, limits the precision of transmission cluster identification. Future longitudinal, multi-genomic region studies with integrated clinical and behavioral data are needed to fully characterize resistance evolution in this population

## CONCLUSIONS

This study reveals a critical public health concern: a high prevalence of acquired HIV drug resistance (31,3 %) among gay and bisexual men on antiretroviral therapy in Surabaya, Indonesia, far exceeding the WHO threshold of 10 % for intervention. The near-ubiquitous dominance of CRF01_AE, coupled with the emergence of recombinant forms and multi-class resistance mutations—including K65R, K103N, and major PI mutations—underscores the evolving complexity of the HIV epidemic in this key population. These findings provide strong evidence that the current one-size-fits-all ART approach is insufficient to sustain long-term viral suppression in high-risk urban networks. To preserve treatment efficacy and prevent the further spread of resistant virus, Indonesia must urgently integrate routine genotypic resistance testing into its national HIV program and transition to more robust, resistance-resistant regimens, such as integrase inhibitor-based therapies. Without such strategic scale-up of precision medicine and targeted surveillance, the goal of ending AIDS as a public health threat by 2030 remains at risk.

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## CONFLICT OF INTEREST

None.

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